

## TWO DIFFERENT MANIFESTATIONS OF ENHANCEMENT IN THE PHOTOSYNTHESIS OF *PORPHYRIDIUM CRUENTUM* IN FLASHING MONOCHROMATIC LIGHT\*

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**Abstract**—Oxygen evolution from *Porphyridium cruentum* exposed to monochromatic light flashes with background light of different wavelengths and intensities was measured. Two different manifestations of the enhancement effect were found: (1) Type I (also referred as  $E_H$ ): an increase in the rate of  $O_2$  evolution and (2) Type II (also referred as  $E_I$ ): a prolongation of  $O_2$  evolution in time. Exposure of algae to red flashes with green background light revealed both types of enhancement whereas green flashes with red background light revealed only the first type of enhancement. Similar results were observed under both aerobic and anaerobic conditions except that in the latter, complications arise due to S-shape of the curve of rate of photosynthesis versus light intensity in a nitrogen atmosphere.

The results presented in this paper and those presented in the earlier papers of French have been interpreted in terms of the 'spill-over' and the 'separate package' model for the distribution of light energy into the two pigment systems. In both the models, we incorporate the idea that the product of light reaction I is long-lived (half-life of the order of seconds). No assumption is needed about the life time of the photoproduct of reaction II in the spill-over model; in the separate package model, however, we have to incorporate an additional assumption that photoproduct II is short-lived (half-life less than 4 msec) to explain all the results.

### INTRODUCTION

SINCE Robert Emerson's discovery<sup>(2,5)</sup> that photosynthesis requires two light reactions, the nature of these reactions has been studied intensively.‡ Except for the recent work of Whittingham<sup>(22)</sup> and French,<sup>(9)</sup> no study of the kinetics of  $O_2$  evolution in flashing monochromatic light has been made. This method will undoubtedly continue to contribute significantly to our understanding of the photochemistry of photosynthesis.

The importance of flashing light experiments in kinetic studies of photosynthesis is well known; the concept of the 'photosynthetic unit' has evolved<sup>(12)</sup> from the flashing light experiments of Emerson and Arnold.<sup>(3)</sup> Recent experiments of Witt and coworkers<sup>(21)</sup> on difference spectra of whole cells and chloroplasts after exposure to light flashes ( $10^{-5}$  to 1 sec), were used by them for a detailed analysis of the two light reactions mechanism of photosynthesis.

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‡See articles by various authors in *Photosynthetic Mechanisms in Green Plants*, Publication 1145 of the National Academy of Sciences—National Research Council, Washington, D.C.; Symposium held at the Airlie House, Warrenton, Virginia, October, 1963.

That the cooperation of these two reactions may involve stable intermediate photo-products, was first suggested by Emerson's early<sup>(5)</sup> experiments in which he observed enhancement even when two beams of light entered a dense cell suspension from two sides of a continuously shaken reaction vessel, so that cells exposed to one beam received the other beam only after a certain interval of darkness. The experiments, of Myers and French<sup>(18)</sup> showed that enhancement effects must involve very stable intermediates; they observed enhancement even after a 6 sec interval between exposures to the two beams. This eliminated purely physical interpretations of the effect, and called for a chemical mechanism.

French<sup>(10)</sup> recently reviewed his observations, showing interesting differences in the kinetics of photosynthesis of red algae, in red light (exciting pigment system I preferentially) and green light (exciting pigment system II preferentially), using flashes of 4 msec – 3 sec. duration.

In confirmation of French's observations, we found two manifestations of enhancement in the photosynthesis of *Porphyridium cruentum* (a) Type I, an increase in the initial rate of O<sub>2</sub> evolution caused by a 50 msec flash of green light imposed on a red-light background; and (b) Type II, a prolongation of oxygen evolution caused by red-light flashes when imposed on a green-light background. We report here a systematic study of the O<sub>2</sub> evolution from *P. cruentum* exposed to monochromatic light flashes with background light of different wavelengths and intensities.

## MATERIALS AND METHODS

### 1. The organism

*Porphyridium cruentum*, a red alga, was chosen for this study because in it the two pigment systems I and II are spectrally well separated. Phycoerythrin, the bulk pigment of system II absorbs green light (ca. 545 m $\mu$ ), while chlorophyll *a*, the bulk pigment of system I, absorbs red light (ca. 680 m $\mu$ ) and blue light (ca. 436 m $\mu$ ). *Porphyridium* was grown in artificial sea-water,\* centrifuged out of its culture medium and spread as a thin paste on a teflon-covered platinum electrode.

### 2. The polarographic apparatus

O<sub>2</sub> evolution was measured with a polarized platinum electrode-silver chloride cell.<sup>(11)</sup> A potential of -0.6 V was applied to the platinum terminal. A dialysis membrane was placed above the algal paste.<sup>(6)</sup> The culture-medium, contained in a large reservoir, was pumped by means of an electrically insulated pump,<sup>(16)</sup> over this membrane. This kept the algae in healthy condition.

### 3. The optical system

Two tungsten lamps were used as light sources. Monochromatic light of appropriate wavelength was obtained by means of interference filters, combined with colored-glass filters (see Table 1). Because the light beams impinging on the interference filters were not exactly parallel, the transmission maxima were shifted by about 5 m $\mu$  towards the shorter wavelengths, and the half band-widths were somewhat larger than the indicated 10 m $\mu$ .

\*The following is the medium (artificial sea water) used in Dr. French's laboratory for growing *Porphyridium* (all figures within brackets except when otherwise stated, are in g/l): NaCl(32.6), MgSO<sub>4</sub> · 7H<sub>2</sub>O(6.66), MgCl<sub>2</sub> · 6H<sub>2</sub>O(5.49), CaCl<sub>2</sub>(1.11), KNO<sub>3</sub>(1.01), KH<sub>2</sub>PO<sub>4</sub>(0.068), NaHCO<sub>3</sub>(0.042); ZnCl<sub>2</sub>(0.004 mg/l), (NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub> · 4H<sub>2</sub>O(0.0368 mg/l), CuCl<sub>2</sub> · 2H<sub>2</sub>O(0.0034 mg/l), MnCl<sub>2</sub> · 4H<sub>2</sub>O(0.04 mg/l), H<sub>3</sub>BO<sub>3</sub>(0.06 mg/l) and FeCl<sub>3</sub> · 6H<sub>2</sub>O in 0.05M NaEDTA(2.4 mg/l).

TABLE 1. FILTERS AND RELATIVE LIGHT INTENSITIES

Transmission Maxima of the Filters, $\mu$	Coloured Glass used in Combination with Interference Filters	Relative Intensities Measured by a Thermopile
700 (Baird and Atomic High Transmission Filter)	Corning 2403	1000
694 (Bausch and Lomb)	Corning 2403	60
556 (Bausch and Lomb)	Corning 3486	30
550 (Bausch and Lomb)	Corning 3484	50

The two beams, originally at  $180^\circ$  to each other, passed through water filters and were then focused, by means of appropriate lenses and mirrors, on the same place on the electrode. Light intensity was varied by wire screens and was measured by replacing the electrode assembly with a thermopile.

Light flashes were obtained by chopping the beams by a rotating sector. We have used only 50 msec flashes. Long dark periods, of the order of 20 sec and greater, were interposed between flashes.

#### 4. The exposure of the sample

First, *Porphyridium* was exposed to flashes without any background light. The electrical signal generated as a consequence of  $O_2$  evolution or uptake by the alga was amplified by a Beckman amplifier and displayed on a Nesco recorder (chart speed 12 in/min). A typical recording of  $O_2$  evolution from 50 msec flashes is shown in Fig. 1. The height,  $H$ , is a

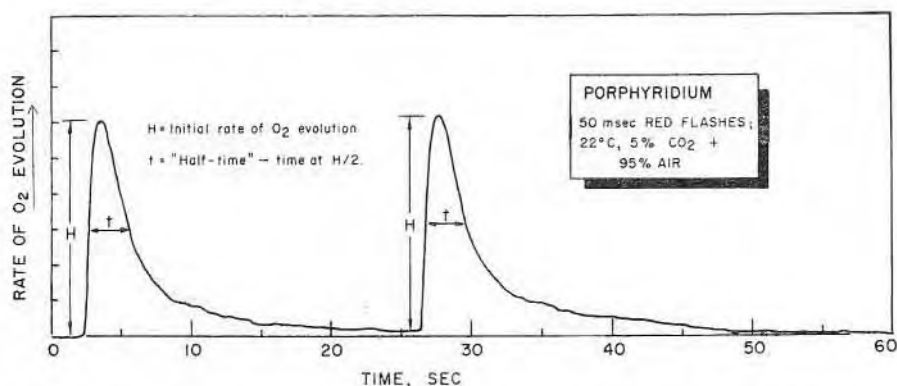


FIG. 1. Rate of  $O_2$  evolution in *Porphyridium Cruentum* induced by 50 msec red (694  $\mu$ ) flashes as a function of time.  $22^\circ C$ , 5%  $CO_2$  in air. Flashes given at 25 sec intervals.

measure of the maximum rate of  $O_2$  evolution in the flash. The time,  $t$ , is the width of the trace at one-half of the height,  $H$ , and is referred to as the half-time. Its duration is partly

due to the instrument (and diffusion) lag but any increase in half-time results from a prolongation of  $O_2$  evolution after the flash. After at least three exposures to flashes without background light, the sensitivity of the instrument and the chart speed were reduced, and continuous background light of complementary colour was turned on. The rate of photosynthesis due to this light was measured. The constant signal was then electrically compensated, the chart speed increased, and then light flashes were superimposed on the background illumination. The experiments were repeated with various intensities of background light. To check for possible artifacts due to non-linearity of light curves, red flashes were also superimposed on red background light, and green flashes on green light of different intensity. Experiments were made at room temperature ( $22^\circ C$ ) and at low temperature ( $3-5^\circ C$  obtained by circulating cold culture medium). In most experiments a mixture of 5%  $CO_2$ - and 95% air was bubbled through the large reservoir of the circulating medium. Anaerobic conditions were obtained by bubbling a mixture of 5%  $CO_2$  and 95% nitrogen through the culture medium.

## RESULTS

### 1. Rate of $O_2$ -evolution as function of light intensity

True enhancement can be inferred only if the curve of photosynthesis rate versus light intensity (referred as 'light curve') is known.<sup>(13)</sup> This called for the measurement of these curves under conditions of the experiments.

At room temperature and under aerobic conditions (5%  $CO_2$  in air), the light curves were found to be linear in both the 556 and the 700  $m\mu$  light in the lower intensity

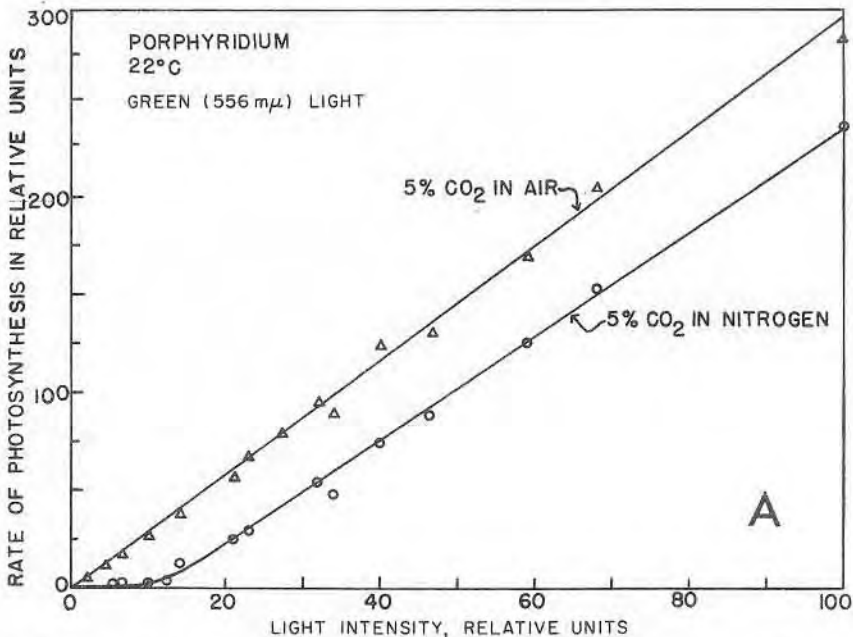


FIG. 2a. Rates of photosynthesis as a function of green (556  $m\mu$ ) light intensity in aerobic (5%  $CO_2$  + 95% air; open triangles) and anaerobic (5%  $CO_2$  + 95% nitrogen; open circles) conditions. Note the concave shape of 'light curve' in anaerobic conditions at low light intensities.

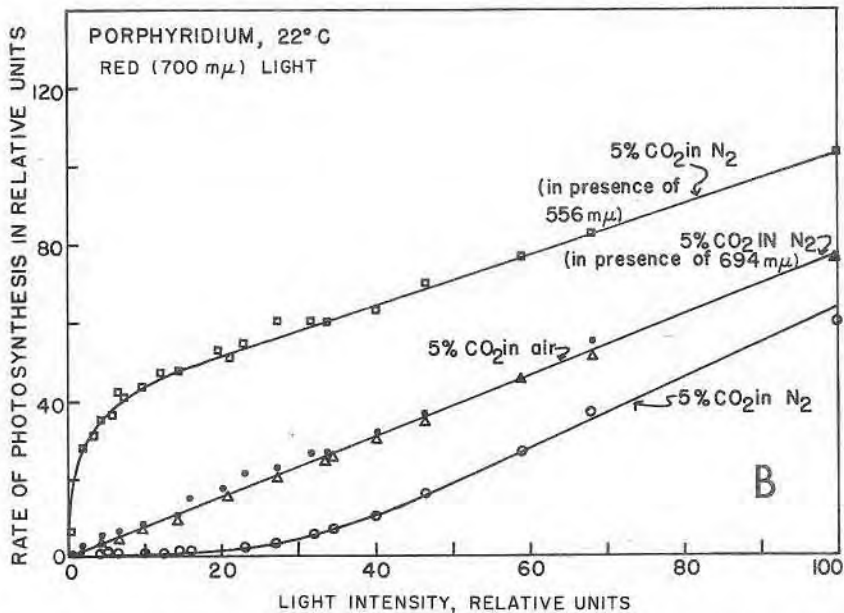


FIG. 2b. Rates of photosynthesis as a function of red ( $700\text{ m}\mu$ ) light intensity in aerobic and anaerobic conditions, in presence and absence of supplementary lights. Open triangles: aerobic; open circles: anaerobic; black dots: rate of photosynthesis in combined  $700\text{ m}\mu$  and  $694\text{ m}\mu$  beams given together minus rate in  $694\text{ m}\mu$  beam under anaerobic condition; open squares: rate of photosynthesis in combined  $556\text{ m}\mu$  and  $700\text{ m}\mu$  beams given together minus the rate in  $556\text{ m}\mu$  beam.

range (open triangles in Figs. 2a and 2b). The light curves for red and green flashes also were approximately linear; although a slight deviation from linearity may exist at very low light intensities.

Under anaerobic conditions (5%  $\text{CO}_2$  + 95% nitrogen), the light curves at room temperature are non-linear in the low-intensity range at both  $556$  and  $700\text{ m}\mu$  (open circles in Figs. 2a and 2b.)

The S-shape of a light curve under anaerobic conditions could be due either to a depressing narcotic effect of nitrogen on photosynthesis (explanation which Franck, *et al.*,<sup>(7)</sup> suggested for the S-shaped curve they had observed in white light under anaerobic conditions) or more simply to immediate re-absorption of oxygen produced by photosynthesis. As the light intensity is increased, the cells produce more and more oxygen; after the rate of  $\text{O}_2$  uptake had become equal to that of  $\text{O}_2$  evolution, the rate of net  $\text{O}_2$  evolution begins to rise linearly with intensity. The light curve obtained at  $700\text{ m}\mu$  under anaerobic condition becomes linear when measured in the presence of another red beam ( $694\text{ m}\mu$ ), as shown by black dots in Fig. 2b. The difference between the lower curve (open circles) and the curve with black dots shows the extent of spurious enhancement. In the presence of a  $556\text{ m}\mu$  beam, (open squares in Fig. 2b), the curve rises steeply and then becomes parallel to the middle curve. Real Emerson enhancement is indicated by the difference between the upper and the middle curve.

At low temperature ( $3\text{--}5^\circ\text{C}$ ) and under aerobic conditions, the light curve at  $556\text{ m}\mu$  is linear; but at  $700\text{ m}\mu$  the light curve shows a break. The  $700\text{ m}\mu$  curve shows no true

saturation at low intensity as previously suggested by ourselves and by others; instead, the light curve for  $700\text{ m}\mu$  continues to rise slowly and seems to approach ultimately the same saturation level as at  $556\text{ m}\mu$ <sup>(13)</sup>. This break in the light curve (Kok effect) could be due to the effect of light on oxygen uptake.<sup>(14,17)</sup> The negative Emerson effect<sup>(15)</sup> may be due to this break.<sup>(17)</sup> The light curves for red and green flashes at low temperature show that only a very short linear portion is available under these conditions for the measurement of enhancement (Fig. 3).

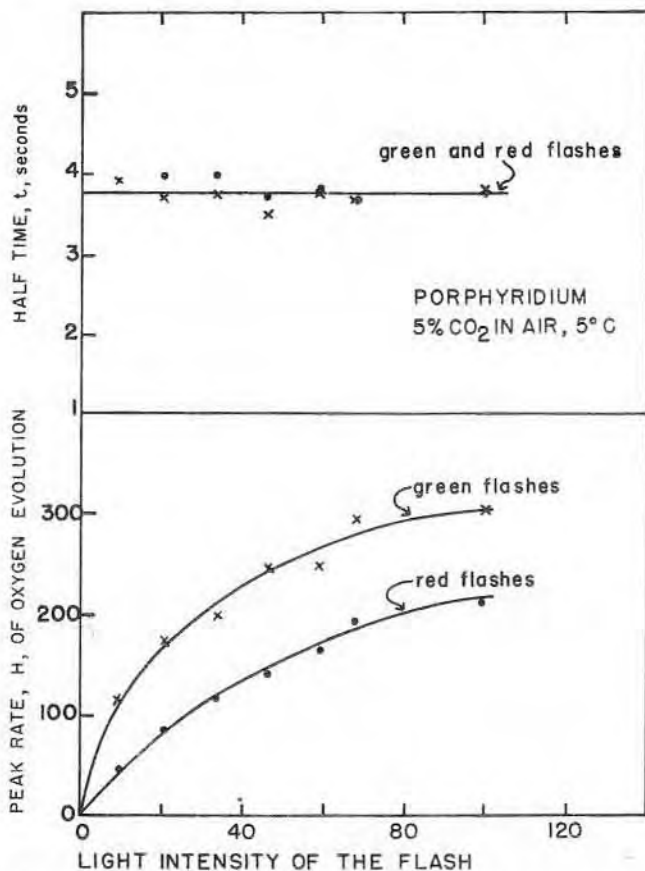


FIG. 3. Half-time,  $t$ , of decay of  $\text{O}_2$  evolution and rate of  $\text{O}_2$  evolution,  $H$ , by a flash as a function of intensity of light flashes measured at  $5^\circ\text{C}$ . Note the bending light curves; at room temperature these curves were linear at the same intensities.

With this information on the shape of light curves on hand, we proceeded to measure the effect of background light of varying intensity on the initial rate of  $\text{O}_2$  evolution  $H$  and the half time  $t$ .

## 2. Red flashes on green background light (aerobic conditions; room temperature)

With increasing intensity of the background light two effects on the rate of  $\text{O}_2$  evolution are observed. The period of  $\text{O}_2$  evolution is prolonged and the maximum rate increases to an optimum. At still higher background intensity both effects decrease from their maxima.

*Prolongation of O<sub>2</sub> evolution.* Two comparable experiments were done with red (700 m $\mu$ ) flash of intensity  $I_1$  and  $I_2$  ( $I_2 > I_1$ ). Figure 4 shows that the half time,  $t$ , of oxygen production by a red flash of intensity  $I_1$  (curve with crosses) increased ( $t = 4.0$  sec.) with increasing intensity of green background light; the effect then declined. The higher intensity red flash ( $I_2$ ; curve with open circles) gave a smaller increase in the half time of O<sub>2</sub> evolution ( $t = 3.2$  sec.) than did the lower intensity  $I_1$ .

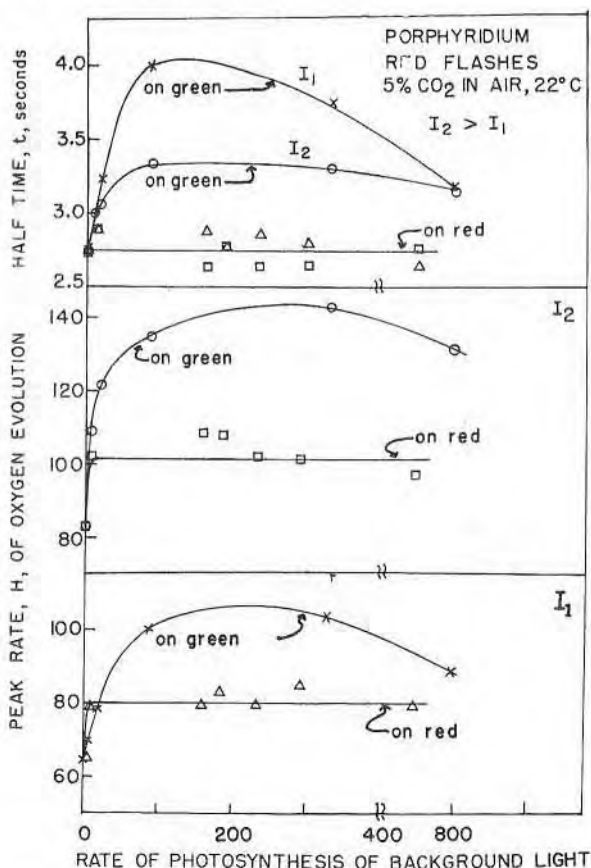


FIG. 4. Half-time,  $t$ , of decay of O<sub>2</sub> evolution and peak rate,  $H$ , of O<sub>2</sub> evolution from red flashes (ca. 964 m $\mu$ ) of two intensities  $I_1$  and  $I_2$  ( $I_2 > I_1$ ) as a function of the rate of photosynthesis in green (ca. 550 m $\mu$ ) background light. When red flashes are given on different intensities of green background light two different manifestations of enhancement are seen: (a) Type I (or  $E_H$ ) by the increase in peak rate,  $H$  (shown in the lower curves) and (b) Type II (or  $E_t$ ) by prolongation of the time,  $t$ , of O<sub>2</sub> evolution (shown in the top curves).

In a separate series of measurements shown in Fig. 5, the shape of the curve relating the half-time to the green background intensity was studied in more detail. This experiment showed the greatest increase in  $t$  ( $t = 8$  sec). No prolongation of  $t$  was observed when red flashes of intensity  $I_1$  or  $I_2$  were superimposed on red (694 m $\mu$ ) background light of different intensities. This confirms beyond doubt that prolongation of O<sub>2</sub> evolution after a flash is a real effect. The extent of prolongation of O<sub>2</sub> evolution depends on the intensity of the flash as well as on the intensity of background light.

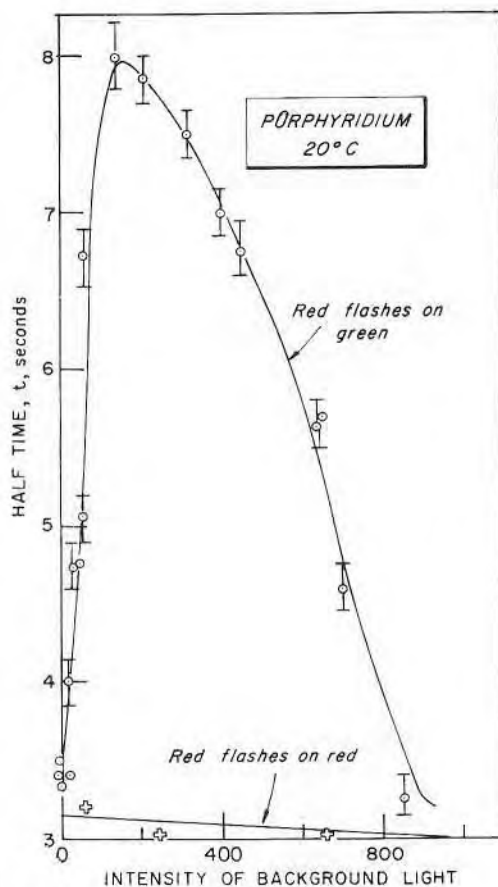


FIG. 5. Half-time,  $t$ , of decay of  $O_2$  evolution by red flashes as a function of the intensity of light 20°C, 5%  $CO_2$  in air.

On the basis of changes in  $t$ , an enhancement factor can be calculated. This factor will be referred to as enhancement of type II or simply  $E_t$ . For the red flash, it may be defined as:

$$E_{t_1} = \frac{t_{1,2}}{t_{1,1}} \quad (1)$$

in which  $t_{1,2}$  is the half-time by the red flash imposed on green background light and  $t_{1,1}$  is the half-time by the same flash imposed on red background light of equivalent intensity. Similarly for green flash,  $E_{t_2} = t_{2,1}/t_{2,2}$ . Figure 6 (graph on the right) shows the general shape of the curve obtained for  $Et$  versus rate of photosynthesis in background light. In the linear range of 'light curve', the latter is equivalent to the intensity of background light.

*Changes in the initial rate of  $O_2$  evolution.* The middle and the bottom curves in Fig. 4 show a plot of the rate of oxygen evolution by a flash, measured by height,  $H$ , as a function of the intensity of background light. In spite of the linearity of the light curves in constant light, a small spurious enhancement was always noted under aerobic conditions, when red flashes were superimposed on red background light. This artifact could be due, at least in



part, to a slight inflection of the light curve at very low light intensities (caused perhaps by a partial anaerobic condition prevailing at the electrode, in spite of the bubbling of a  $\text{CO}_2/\text{air}$  mixture through the culture medium).

A true enhancement by a factor of about 1.4 was noted (compare the 'red on red' curves with 'red on green' curves for red flashes of intensity  $I_2$ ). This enhancement, which is an enhancement in the initial rate of  $\text{O}_2$  evolution, will be referred to as enhancement of type I or simply  $E_H$ . For the red flash, it may be defined as:

$$E_{H_1} = \frac{H_{1,2}}{H_{1,1}} \quad (2)$$

in which  $H_{1,2}$  is the initial rate by the red flash imposed on green back-ground light and  $H_{1,1}$  is the rate by the same flash imposed on red background light. Similarly for green flash,  $E_{H_2} = H_{2,1}/H_{2,2}$ . Figure 6 (graph on the left) shows the general shape of the curve obtained for  $E_H$  versus intensity of background light.

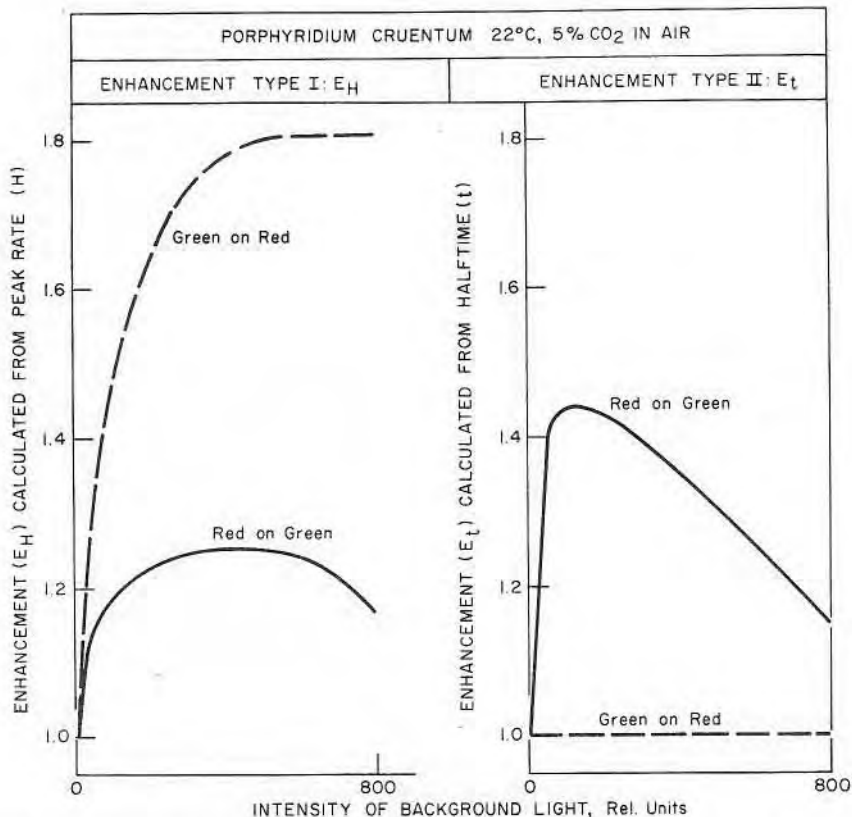


FIG. 6. Curves relating the two different manifestations of enhancement (Type I or  $E_H$  and Type II or  $E_t$ ) to different intensities of background light. Enhancement of type I is due to an increase of the peak height and enhancement of type II is due to prolongation of the time during which  $\text{O}_2$  is evolved (see text for description).

Enhancement,  $E_H$ , in the rate of  $O_2$  evolution from a flash increases with increasing intensity of green background light; the effect then saturates, and shows a decline at the highest intensity used.

### 3. Green flashes on red background (aerobic conditions; room temperature)

*Half-time.* In contrast to the effect of red flashes on a green background no prolongation of the  $O_2$  evolution period was noticed at any intensity of red background light used with green flashes (top curve in Fig. 7 and lower right curve on Fig. 6).

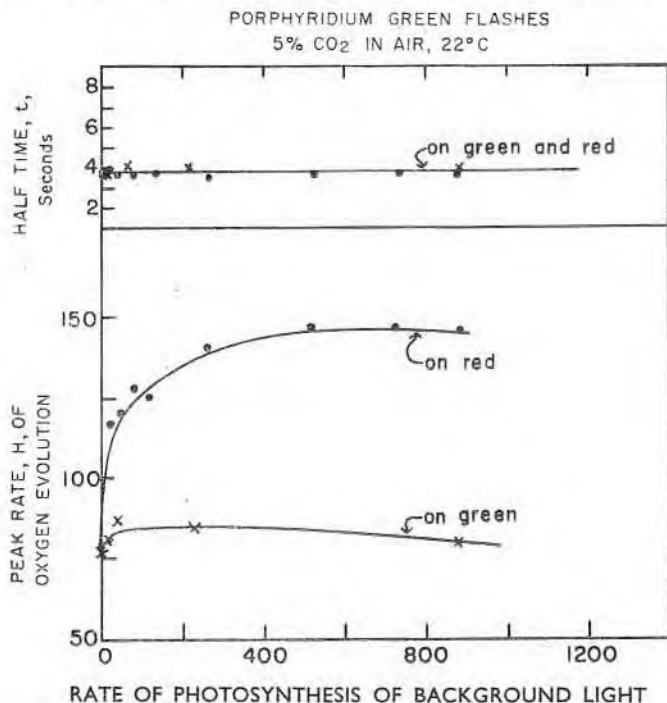


FIG. 7. Half-time,  $t$ , of decay of  $O_2$  evolution and rate of  $O_2$  evolution,  $H$ , by green flashes as a function of rate of photosynthesis in either green or red background light. 22°C, 5%  $CO_2$  in air.

*The initial rate.* The peak rate of  $O_2$  evolution from a green flash on red background light first increases with an increase in the intensity of red background light, and then attains saturation at an enhancement ( $E_H$ ) of the order of 1.8 (bottom curve, Fig. 7 and upper left curve on Fig. 6). A small, spurious enhancement was noted when green flashes were superimposed on green background light.

### 4. Red flashes on green background (aerobic conditions; low temperature)

These flashes added to green background light of different intensities showed no enhancement at all at low temperature (3–5°C). A spurious increase was noticed, however, when red flashes were given on red background light (see Table 2). These observations, suggestive of a negative Emerson effect, may be due—as mentioned before—to an effect of illumination on the oxygen uptake—an interpretation which could be tested only means of a

TABLE 2. LOW TEMPERATURE EXPERIMENTS - RATE OF O<sub>2</sub> EVOLUTION IN RELATIVE UNITS

Background		Green Flashes	Background		Red Flashes
m $\mu$	Intensity, Relative Units	(550 m $\mu$ ) 'H'	m $\mu$	Intensity, Relative Units	(694 m $\mu$ ) 'H'
Dark	—	88	Dark	—	72
694	2.0	128	700	35	96
694	4.5	96	700	90	100
694	9.0	88	700	185	88
556	2.0	100	556	2.0	80
556	4.5	88	556	4.5	80
556	9.0	48	556	9.0	60

mass-spectrometer, permitting measurement of oxygen uptake simultaneously with oxygen liberation by photosynthesis. We did not observe any prolongation of O<sub>2</sub> evolution, (change in decay time  $t$ ) at any of the intensities used.

#### 5. Green flashes on red background (aerobic conditions; low temperature)

A real enhancement was noted in the low-intensity range when a green flash was given on red background light of different intensities. Green flash on green background light produced an insignificant spurious increase (see Table 2). No change in the half-time of decay was noted at any of the intensities employed.

Emerson *et al.*,<sup>(4)</sup> have shown that the quantum yield of photosynthesis in far red light is higher at 5°C than at 20°C. Thus, lesser enhancement is expected at 5°C than at 20°C. As shown above, we did not observe any real enhancement at 5°C with red flashes on green background light at the intensities we have used. A spurious enhancement was noted when red flashes were combined with red background light. Inhibitions observed at higher intensities are obviously due to saturation effects, since the saturation occurs at lower intensities at these low temperatures.

The higher quantum yield in far red light<sup>(4)</sup> and lowered enhancement at low temperatures may be explained if we postulate that lowering of temperature transfers some chlorophyll *a* in system I to system II. Such a transfer is not inconceivable, if the stability of chlorophyll complexes is assumed to be temperature-dependent. Since techniques so far employed cannot unequivocally distinguish between O<sub>2</sub> uptake and O<sub>2</sub> evolution in light, a reduced O<sub>2</sub> uptake in red light at low temperature will also explain the low temperature results.

#### 6. Red flashes on green background light (anaerobic conditions; room temperature)

When a mixture of 5 per cent CO<sub>2</sub> with 95% nitrogen was bubbled through the culture medium, we called it 'anaerobic' condition—although truly anaerobic conditions cannot persist in strong light, when photosynthesis causes liberation of oxygen.

Due to non-linear shape of the light curves under anaerobic conditions, a control experiment, using red flashes on red background, had to be made. In addition to the spurious enhancement estimated from this experiment, red flashes on green background light showed also true enhancement. In the low-light range, no increase in the rate of O<sub>2</sub> evolution could be identified in this particular experiment. A true prolongation of the O<sub>2</sub> evolution period, superimposed on a spurious prolongation, was noticed when red flashes were given on green background (Fig. 8). As yet we do not understand why a spurious increase in  $t$  was noticed here. Qualitatively, these results confirm those obtained under aerobic conditions.

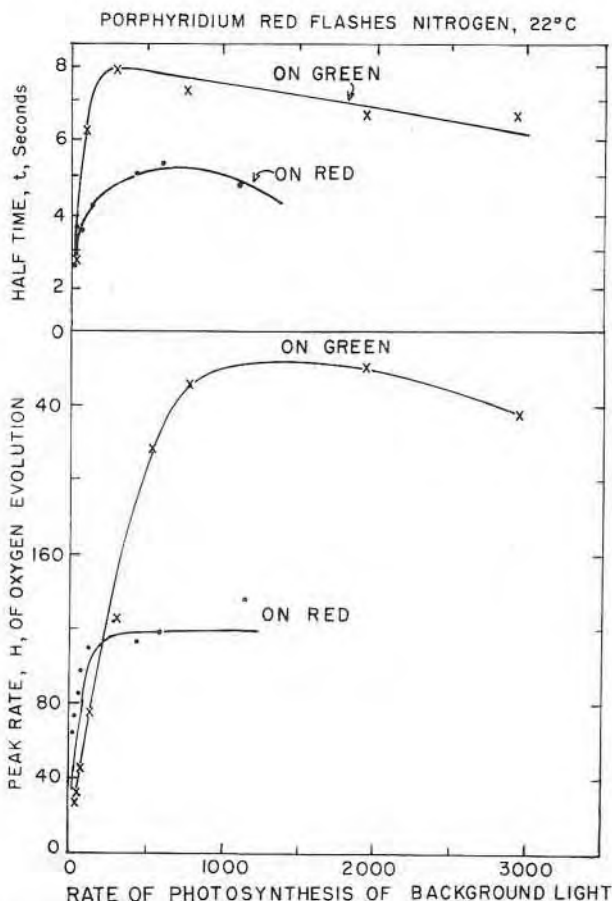


FIG. 8. Half-time,  $t$ , of decay of  $O_2$  evolution and rate of  $O_2$  evolution,  $H$ , by red flashes as a function of the rate of photosynthesis in background light under 'anaerobic' conditions. Note the spurious effect due to non-linear light curves from the red flashes on red background, 22°C, 5%  $CO_2$  in  $N_2$ .

#### 7. Green flashes on red background light ('anaerobic' conditions; room temperature)

A true enhancement of the order of 2.0 was calculated when green flashes were given on red background light (Fig. 9). A spurious enhancement (green flashes on green background), due to non-linear shape of the light curves, also was noted. No change in  $t$  was observed. These results confirm the findings obtained under aerobic conditions.

### DISCUSSION AND INTERPRETATIONS

Most recent hypotheses concerning the photochemical stage of photosynthesis are based on the assumption that there are two consecutive light reactions. The enhancement observed (Fig. 4-9), by combining monochromatic flashes with continuous monochromatic light, supports this concept. In their magnitude and their dependence on light intensity, the enhancements reported in this paper are similar to those observed with two beams of continuous light.<sup>(13)</sup>

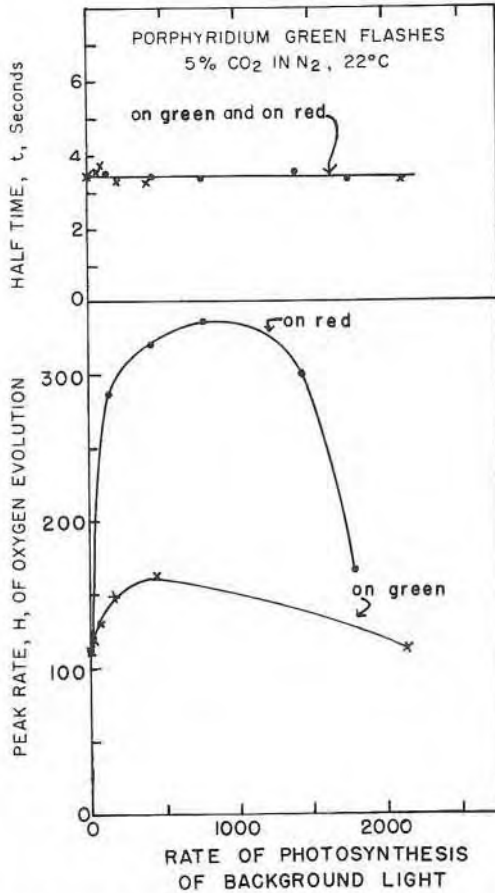


FIG. 9. Half-time,  $t$ , of decay of  $O_2$  evolution and rate of  $O_2$  evolution,  $H$ , by green flashes as a function of rate of photosynthesis in background light under 'anaerobic' conditions. Note the spurious effect due to non-linear light curves from the green flashes given on green background,  $22^\circ C$ ,  $5\% CO_2$  in  $N_2$ .

Two interesting phenomena<sup>(10)</sup> are (1) the increase in the yield from a green flash if it is preceded by a red flash, and the absence of a similar increase in the yield from a red flash when preceded by a green flash, and (2) prolongation of  $O_2$  evolution due to a red flash, if it is given on a background of green light; and absence of such a prolongation if a green flash is given on a background of red light! Both phenomena can be interpreted in the two-light reaction scheme of photosynthesis, either in terms of 'spillover' or the separate 'package' mechanism (Myers and Graham<sup>(19)</sup> discuss the definition and the origin of the two mechanisms).

#### A. Spill-over model

In the spill-over model, we must make one assumption: that the product of light reaction I is long lived (half-life of the order of seconds); no assumption is needed about the life time of photoproduct II.

According to this model,<sup>(8,19)</sup> light energy absorbed in system II can be transferred by resonance to system I (energy spills over from system II to system I, but not vice versa), leading to balanced excitation of the two systems when light is predominantly absorbed by system II. Just how this comes about is uncertain; it is energetically possible because system I absorbs light of longer wavelengths than system II.

If a green flash, which is mainly absorbed in system II, is used to illuminate the red alga, *Porphyridium cruentum*, both systems I and II are excited according to this picture in a balanced way; all light products are used for photosynthesis, and no excess product of any kind is left over. Therefore a red flash produces the same amount of oxygen with or without a preceding green flash. On the contrary, a red flash excites mainly system I in *Porphyridium*. Therefore, in red flash, light reaction I runs 'in excess'. If we assume that the product of this light reaction survives for seconds, a green flash preceded by a red flash can produce more oxygen—its quanta can be now used entirely for reaction II, and do not have to be distributed between the two systems. French's earlier results<sup>(10)</sup> on the interaction of monochromatic flashes are thus explained. This explanation was first given by Rabinowitch<sup>(20)</sup> in the discussion at the Symposium on Photosynthesis held at the Airlie House in October, 1963.

It is now shown that the spill-over hypothesis can explain also the interaction of flashing light with continuous light observed in *Porphyridium*. In green background light, systems I and II are equally excited, in consequence of spill-over. When a red flash is given on green background light (Figs. 4–6), the green quanta are distributed during the flash, in a different way than before (more of them can stay in system II), thus causing enhancement. Depending upon the relative intensities of green background light and the red flash, one can anticipate a larger or a smaller enhancement in O<sub>2</sub> evolution by the flash. If the red-light product (Product I) is not used up entirely by green light during the flash, or as will be the case when the intensity of green background light is low, the green light arriving after the flash can continue producing excess oxygen, until all the long-lived product had been used up. Thus, the enhancement effect will continue for some time after the red flash is over. This explains the prolongation of O<sub>2</sub> evolution observed when a red flash is given on green background light. With green background light of higher intensity, the excess red light product will be used up more quickly, explaining why the prolongation of O<sub>2</sub> evolution becomes shorter and ultimately disappears (Figs. 5,6). Nevertheless, enhancement will still be revealed by a greater rate of O<sub>2</sub> evolution (Figs. 4,6).

In the other set of experiments (Fig. 7) the red background light creates a photostationary excess of long-lived red light product. If now a green flash is added, the quanta of this flash will utilize this product, and an enhancement of the initial rate of O<sub>2</sub> evolution will result. The extent of this enhancement will depend on the intensity of the red background light. There can be, no prolongation of O<sub>2</sub> evolution due to a green flash, because as soon as the flash ends the photostationary state is reestablished. No intermediate survives a green flash, whether it be long-lived or short-lived, because it is all used up during the flash.

### B. *Separate-package model*

According to this model<sup>(1,19)</sup> light quanta absorbed in one system are not transferred to the other, although both systems contain the same assortment of pigments, even if in different proportions—e.g. system I contains chlorophyll *a* as the major, and accessory pigments as the minor component, whereas system II contains accessory pigments as the major component, and chlorophyll *a* as the minor one.

As in the spill-over model, we postulate that the product of light reaction I has a long life (of the order of seconds); in addition we assume that the product of light reaction II is short-lived (life time less than 4 msec). This explains why French had observed enhancement of the yield of green flash when preceded by a red flash, but not of the yield of red flash when it was preceded by a green flash. Here also the prolongation of  $O_2$  evolution when red flashes are given on green background will be due to the long life of product 'I', making possible its utilization by excess green light. In the reverse experiment, a green flash utilizes more efficiently its light quanta, if it is given on red background, because of the excess green light product (Product II), formed in the green flash, can react with excess red light product, (Product I) provided by red background light. As soon as the green flash ends, no further reaction is possible due to the postulated short life (less than 4 msec) of green light product (Product II), so that no prolongation of  $O_2$  evolution is observed.

The flashing light experiments of French and those presented in this paper can thus be interpreted in terms of both spill-over and separate package models.

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